My name is Dennis Alsop, and I am here today to encourage you to support the dispensary initiative because patients need to be able to access Medical Cannabis quickly when diagnosed with a life threatening condition.

In 2006 I was diagnosed with Bladder Cancer. As recommended by my urologist, I followed traditional medicines protocol for the treatment of bladder cancer. I had the recurring tumors surgically removed for several years. And then as recommended, I then underwent another surgery to remove 3 more tumors which was immediately followed by one intra-vessel chemo treatment. This was a bladder instillation of the chemotherapy drug Mitomycin. I specifically asked the doctor what side effects were to be expected from this treatment and was reassured that there would be NO side effects since it was put directly into the bladder.

The treatment all but destroyed what used to be my normal functioning bladder. The first 3 days following the procedure I had to void every five minutes. I went 9 days without sleeping due to the horrific pain I now had to endure. The pain was indescribable, I lost 20 lbs in 9 days and I seriously thought I was going to die from a treatment that had no side effects.

Worse yet, the doctors in the office that did the treatment did not believe that I was in such pain and agony. They treated me like a "drug seeker" when I asked for something to help relieve the pain. They told me to take Tylenol. They left me with nothing, not even a comforting word.

When I was able to travel I went to a Urologist/Oncologist near Detroit. After an exam I was told that the damage was so severe that there was literally nothing medically that they could even suggest to repair the damage. There was so much scar tissue from the burns that his bladder would now only hold 150ml, which is slight less than 3/4 cup. He went on to say that the cancer could recur beneath the scar tissue and easily spread throughout my body. 1/2

I went home severely depressed. My life as I knew it had been destroyed, I was in continuous horrific pain, I could not control my bladder, I could not work, I rarely left home because I had to be able to use a bathroom, and no one could help me. The pain was making me insane, I thought of ending my life just to make the pain stop.

We decided we had to find something to help heal the tissue and reduce the pain. My wife and daughters did constant research trying to find something, anything that would work. We tried things like aloe juice and cornsilk for the intense burning and herbal tinctures for dissolving scar tissue, but were blown away when we found the healing information on cannabis oil.

There is a massive amount of peer reviewed literature on Cannabis that continues to be conducted and published regarding cannabis/thc/cbd (cannabidiol). Research results were promising.

I applied and receive MMM card in January of 2009. I grew my own plants and as soon as possible I began ingesting cannabis.

In March 2011 we went for another follow-up with the oncologist and he stated, "When you came to me, your bladder looked like a mine field, It is drastically improved, what do you think is doing it?" I said it has to be the Cannabis because I'm not super human, to which he replied, whatever you are doing, keep it up.

The scar tissue is minimized and my capacity has increased significantly, and I have had only one low-grade tumor since I started ingesting cannabis.

We later learned that the MSDS sheet for Mitomycin is 7 pages long and disposal of the product is considered "highly caustic hazardous waste".

I survived a horrific traumatic nightmare and if it were not for medical benefits of cannabis, I would not be here to tell my story.

Mitomycin C

sc-3514

Material Safety Data Sheet

Hazard Alert Code Key: EXTREME | HIGH | MODERATE | LOW

Section 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME

STATEMENT OF HAZARDOUS NATURE

CONSIDERED A HAZARDOUS SUBSTANCE ACCORDING TO OSHA 29 CFR 1910.1200.

NFPA

SUPPLIER

Santa Cruz Biotechnology, Inc.
2145 Delaware Avenue
Santa Cruz, California 95060
800.457.3801 or 831.457.3800

EMERGENCY

ChemWatch
Within the US & Canada: 877-715-9305
Outside the US & Canada: +800 2436 2255
(1-800-CHEMCALL) or call +613 9573 3112

SYNONYMS

C15-H18-N4-O5, "azirino(2' , 3' :3, 4)pyrrolo[1, 2-a]indole-4, 7-dione, 6-amino-1, 1a, 2, ", "8, 8a, 8b-hexahydro-8-(hydroxy methyl)-8a-methoxy-5-methyl-, ", carbamate, mutamycin, mytomycin, NCI-C04706, NSC-CO4706, "RCRA Waste No. U010", MMC, Mit-C, Mito-C, "antineoplastic/ antibody."

Section 2 - HAZARDS IDENTIFICATION

CHEMWATCH HAZARD RATINGS

	Min	Max
Flammability:	1	
Toxicity:	4	
Body Contact:	4	
Reactivity:	1	
Chronic:	3	

Min/Nil=0
Low=1
Moderate=2
High=3
Extreme=4

CANADIAN WHMIS SYMBOLS

EMERGENCY OVERVIEW

RISK

May cause CANCER.
May cause heritable genetic damage.
Possible risk of harm to the unborn child.
Very toxic by inhalation, in contact with skin and if swallowed.
Irritating to eyes, respiratory system and skin.

POTENTIAL HEALTH EFFECTS

ACUTE HEALTH EFFECTS

SWALLOWED

■ Severely toxic effects may result from the accidental ingestion of the material; animal experiments indicate that ingestion of less than 5 gram may be fatal or may produce serious damage to the health of the individual.
■ The killing action of antineoplastic drugs used for cancer chemotherapy is not selective for cancerous cells alone but affect all dividing cells. Acute side effects include loss of appetite, nausea and vomiting, allergic reaction (skin rash, itch, redness, low blood pressure, unwellness and anaphylactic shock) and local irritation.

EYE

■ This material can cause eye irritation and damage in some persons.

SKIN

■ Skin contact with the material may produce severely toxic effects; systemic effects may result following absorption and these may be fatal.
■ This material can cause inflammation of the skin oncontact in some persons.
■ The material may accentuate any pre-existing dermatitis condition.
■ Open cuts, abraded or irritated skin should not be exposed to this material.
■ Entry into the blood-stream, through, for example, cuts, abrasions or lesions, may produce systemic injury with harmful effects.
Examine the skin prior to the use of the material and ensure that any external damage is suitably protected.
■ Solution of material in moisture on the skin, or perspiration, may markedly increase skin corrosion and accelerate tissue destruction.

INHALED

■ Inhalation of dusts, generated by the material, during the course of normal handling, may produce severely toxic effects; these may be fatal.
■ The material can cause respiratory irritation in some persons.
The body's response to such irritation can cause further lung damage.
■ Persons with impaired respiratory function, airway diseases and conditions such as emphysema or chronic bronchitis, may incur further disability if excessive concentrations of particulate are inhaled.

CHRONIC HEALTH EFFECTS

■ There is ample evidence that this material can be regarded as being able to cause cancer in humans based on experiments and other information.
■ Long-term exposure to respiratory irritants may result in disease of the airways involving difficult breathing and related systemic problems.
Based on experiments and other information, there is ample evidence to presume that exposure to this material can cause genetic defects that can be inherited.
Results in experiments suggest that this material may cause disorders in the development of the embryo or fetus, even when no signs of poisoning show in the mother.
Limited evidence suggests that repeated or long-term occupational exposure may produce cumulative health effects involving organs or biochemical systems.
Long term exposure to high dust concentrations may cause changes in lung function i.e. pneumoconiosis; caused by particles less than 0.5 micron penetrating and remaining in the lung.
Anti-cancer drugs used for chemotherapy can depress the bone marrow with reduction in the number of white blood cells and platelets and bleeding. Susceptibility to infections and bleeding is increased, which can be life- threatening.
Delayed and cumulative bone-marrow suppression, profound leucopenia and thrombocytopenia may occur within 4-weeks of treatment.
Other side-effects include renal damage and pulmonary reactions, gastrointestinal toxicity, dermatitis, alopecia, fever and malaise. Delayed toxicity following
injection occurs in mice - death may follow from 2 days to 2 weeks after administration.

Section 3 - COMPOSITION / INFORMATION ON INGREDIENTS

NAME	CAS RN	%
mitomycin C	50-07-7	>98

Section 4 - FIRST AID MEASURES

SWALLOWED

· IF SWALLOWED, REFER FOR MEDICAL ATTENTION, WHERE POSSIBLE, WITHOUT DELAY. · Where Medical attention is not immediately available or where the patient is more than 15 minutes from a hospital or unless instructed otherwise:

EYE

■ If this product comes in contact with the eyes: · Immediately hold eyelids apart and flush the eye continuously with running water. · Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids.

SKIN

■ If skin or hair contact occurs: · Immediately flush body and clothes with large amounts of water, using safety shower if available. · Quickly

remove all contaminated clothing, including footwear.

INHALED

· If fumes or combustion products are inhaled remove from contaminated area. · Lay patient down. Keep warm and rested.

NOTES TO PHYSICIAN

■ Treat symptomatically.

For employees potentially exposed to antineoplastic and/ or cytotoxic agents on a regular basis, a preplacement physical examination and history (noting risk factors) is recommended. Periodic follow-up examinations should also be undertaken and should be overseen by a physician familiar with the toxic effects of the substance and full details of the nature of work undertaken by the employee.

Disappears rapidly from the blood with most metabolised in the liver.

Section 5 - FIRE FIGHTING MEASURES

Vapour Pressure (mmHG):	Negligible
Upper Explosive Limit (%):	Not available.
Specific Gravity (water=1):	Not available
Lower Explosive Limit (%):	Not available

EXTINGUISHING MEDIA

· Water spray or fog.
· Foam.

FIRE FIGHTING

· Alert Emergency Responders and tell them location and nature of hazard.
· May be violently or explosively reactive.

When any large container (including road and rail tankers) is involved in a fire, consider evacuation by 800 metres in all directions.

GENERAL FIRE HAZARDS/HAZARDOUS COMBUSTIBLE PRODUCTS

· Combustible solid which burns but propagates flame with difficulty.
· Avoid generating dust, particularly clouds of dust in a confined or unventilated space as dusts may form an explosive mixture with air, and any source of ignition, i.e. flame or spark, will cause fire or explosion. Dust clouds generated by the fine grinding of the solid are a particular hazard; accumulations of fine dust may burn rapidly and fiercely if ignited.

Combustion products include: carbon monoxide (CO), carbon dioxide (CO2), nitrogen oxides (NOx), other pyrolysis products typical of burning organic material.

May emit poisonous fumes.

FIRE INCOMPATIBILITY

■ Avoid contamination with oxidizing agents i.e. nitrates, oxidizing acids,chlorine bleaches, pool chlorine etc. as ignition may result.

PERSONAL PROTECTION

Glasses:
Gloves:
Respirator:
Particulate

Section 6 - ACCIDENTAL RELEASE MEASURES

MINOR SPILLS

■ It is recommended that areas handling final finished product have cytotoxic spill kits available.

Spill kits should include:
· impermeable body covering,
· shoe covers,
· latex and utility latex gloves,
· goggles,
· approved HEPA respirator,
· disposable dust pan and scoop,
· absorbent towels,
· spill control pillows,
· disposable sponges,
· sharps container,
· disposable garbage bag and
· hazardous waste label.

To avoid accidental exposure due to waste handling of cytotoxics:
· Place waste residue in a segregated sealed plastic container.
· Used syringes, needles and sharps should not be crushed, clipped, recapped, but placed directly into an approved sharps container.
· Dispose of any cleanup materials and waste residue according to all applicable laws and regulations e.g, secure chemical landfill disposal.
· Clean up waste regularly and abnormal spills immediately.
· Avoid breathing dust and contact with skin and eyes.
· Wear protective clothing, gloves, safety glasses and dust respirator.
· Use dry clean up procedures and avoid generating dust.
· Vacuum up or sweep up. NOTE: Vacuum cleaner must be fitted with an exhaust micro filter (HEPA type) (consider explosion-proof machines designed to be grounded during storage and use).

· Dampen with water to prevent dusting before sweeping.
· Place in suitable containers for disposal.
All personnel likely to involved in a antineoplastic (cytotoxic) spill must receive practical training in:
· the correct procedures for handling cytotoxic drugs or waste in order to prevent and minimize the risk of spills
· the location of the skill kit in the area.
MAJOR SPILLS
· Clear area of personnel and move upwind.
· Alert Emergency Responders and tell them location and nature of hazard.

Section 7 - HANDLING AND STORAGE

PROCEDURE FOR HANDLING

■ The National Institute of Health (USA) recommends that the preparation of injectable antineoplastic drugs should be performed in a Class II laminar flow biological safety cabinet and that personnel preparing drugs of this class should wear appropriate personal protective gear. Emphasise controls on containment.
· Avoid all personal contact, including inhalation.
· Wear protective clothing when risk of exposure occurs.
Empty containers may contain residual dust which has the potential to accumulate following settling. Such dusts may explode in the presence of an appropriate ignition source.
· Do NOT cut, drill, grind or weld such containers.
· In addition ensure such activity is not performed near full, partially empty or empty containers without appropriate workplace safety authorisation or permit.

RECOMMENDED STORAGE METHODS

■ Glass container.
· Lined metal can, Lined metal pail/drum
· Plastic pail.
For low viscosity materials
· Drums and jerricans must be of the non-removable head type.
· Where a can is to be used as an inner package, the can must have a screwed enclosure.
All inner and sole packagings for substances that have been assigned to Packaging Groups I or II on the basis of inhalation toxicity criteria, must be hermetically sealed.

STORAGE REQUIREMENTS

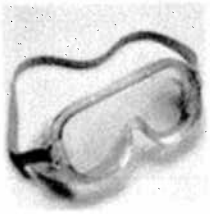
■ Antineoplastics (cytotoxics):
· should be clearly identifiable to all personnel involved in their handling
· should be stored in impervious break-resistant containers.
· Store in original containers.
· Keep containers securely sealed.

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS

The following materials had no OELs on our records
• mitomycin C: CAS:50-07-7

PERSONAL PROTECTION

RESPIRATOR

Particulate
Consult your EHS staff for recommendations

EYE

· Chemical protective goggles with full seal
· Shielded mask (gas-type)
· Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lens or restrictions on use, should be created for each workplace or task. This should include a review of lens absorption and adsorption for the class of chemicals in use and an account of injury experience. Medical and first-aid personnel should be trained in their removal and suitable equipment should be readily available. In the event of chemical exposure, begin eye irrigation immediately and remove contact lens as soon as practicable. Lens should be removed at the first signs of eye redness or irritation - lens should be removed in a clean environment only after workers have washed hands thoroughly. [CDC NIOSH Current Intelligence Bulletin 59].

HANDS/FEET

■ Elbow length PVC gloves.
Suitability and durability of glove type is dependent on usage. Important factors in the selection of gloves include: such as:

· frequency and duration of contact,
· chemical resistance of glove material,
· glove thickness and
· dexterity
Select gloves tested to a relevant standard (e.g. Europe EN 374, US F739).
· When prolonged or frequently repeated contact may occur, a glove with a protection class of 5 or higher (breakthrough time greater than 240 minutes according to EN 374) is recommended.
· When only brief contact is expected, a glove with a protection class of 3 or higher (breakthrough time greater than 60 minutes according to EN 374) is recommended.
· Contaminated gloves should be replaced.
Gloves must only be worn on clean hands. After using gloves, hands should be washed and dried thoroughly. Application of a non-perfumed moisturiser is recommended.

OTHER

· When handling antineoplastic materials, it is recommended that a disposal work-uniform (such as Tyvek or closed front surgical-type gown with knit cuffs) is worn.
· For quantities up to 500 grams a laboratory coat may be suitable.
· For quantities up to 1 kilogram a disposable laboratory coat or coverall of low permeability is recommended. Coveralls should be buttoned at collar and cuffs.
· For quantities over 1 kilogram and manufacturing operations, wear disposable coverall of low permeability and disposable shoe covers.
· For manufacturing operations, air-supplied full body suits may be required for the provision of advanced respiratory protection.
· Eye wash unit.
· Ensure there is ready access to an emergency shower.
· For Emergencies: Vinyl suit.

ENGINEERING CONTROLS

■ Unless written procedures, specific to the workplace are available, the following is intended as a guide:
· For Laboratory-scale handling of Substances assessed to be toxic by inhalation. Quantities of up to 25 grams may be handled in Class II biological safety cabinets *; Quantities of 25 grams to 1 kilogram may be handled in Class II biological safety cabinets* or equivalent containment systems Quantities exceeding 1 kg may be handled either using specific containment, a hood or Class II biological safety cabinet*,
· HEPA terminated local exhaust ventilation should be considered at point of generation of dust, fumes or vapors.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL PROPERTIES

Solid.
Mixes with water.

State	Divided solid	Molecular Weight	334.3
Melting Range (°F)	>680	Viscosity	Not Applicable
Boiling Range (°F)	Not available	Solubility in water (g/L)	Miscible
Flash Point (°F)	Not available	pH (1% solution)	6-8 (0.5%)
Decomposition Temp (°F)	Not Available	pH (as supplied)	Not applicable
Autoignition Temp (°F)	Not available	Vapour Pressure (mmHG)	Negligible
Upper Explosive Limit (%)	Not available.	Specific Gravity (water=1)	Not available
Lower Explosive Limit (%)	Not available	Relative Vapor Density (air=1)	>1
Volatile Component (%vol)	Negligible	Evaporation Rate	Not applicable

mitomycin C

log Kow (Sangster 1997): -0.4

APPEARANCE

Blue-violet crystalline powder; mixes with water, acetone, methanol, butyl acetate, cyclohexanone.

Section 10 - CHEMICAL STABILITY

CONDITIONS CONTRIBUTING TO INSTABILITY

· Presence of incompatible materials.
· Product is considered stable.

STORAGE INCOMPATIBILITY

· Avoid strong acids, bases.
Avoid reaction with oxidizing agents.

For incompatible materials - refer to Section 7 - Handling and Storage.

Section 11 - TOXICOLOGICAL INFORMATION

mitomycin C

TOXICITY AND IRRITATION

MITOMYCIN C:

■ unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances.

TOXICITY	IRRITATION
Oral (rat) LD50: 30 mg/kg	Nil Reported
Intraperitoneal (rat) LD50: 2 mg/kg	
Subcutaneous (rat) LD50: 3.25 mg/kg	
Intravenous (rat) LD50: 3 mg/kg	
Oral (mouse) LD50: 23 mg/kg	
Intraperitoneal (mouse) LD50: 4 mg/kg	
Subcutaneous (mouse) LD50: 7.3 mg/kg	
Intravenous (mouse) LD50: 4 mg/kg	
Intravenous (dog) LD50: 0.72 mg/kg	
Intravenous (rabbit) LD50: 3.4 mg/kg	

■ Asthma-like symptoms may continue for months or even years after exposure to the material ceases. This may be due to a non-allergenic condition known as reactive airways dysfunction syndrome (RADS) which can occur following exposure to high levels of highly irritating compound. Key criteria for the diagnosis of RADS include the absence of preceding respiratory disease, in a non-atopic individual, with abrupt onset of persistent asthma-like symptoms within minutes to hours of a documented exposure to the irritant. A reversible airflow pattern, on spirometry, with the presence of moderate to severe bronchial hyperreactivity on methacholine challenge testing and the lack of minimal lymphocytic inflammation, without eosinophilia, have also been included in the criteria for diagnosis of RADS. RADS (or asthma) following an irritating inhalation is an infrequent disorder with rates related to the concentration of and duration of exposure to the irritating substance. Industrial bronchitis, on the other hand, is a disorder that occurs as result of exposure due to high concentrations of irritating substance (often particulate in nature) and is completely reversible after exposure ceases. The disorder is characterised by dyspnea, cough and mucus production.

Exposure to the material for prolonged periods may cause physical defects in the developing embryo (teratogenesis).

WARNING: This substance has been classified by the IARC as Group 2B: Possibly Carcinogenic to Humans.

Somnolence, ataxia, fibrosing alveolitis, dyspnea, diarrhoea, gastrointestinal tumours, changes in kidney tubules, haemorrhage, normocytic anaemia, haemolysis, skin tumours, paternal effects, effects on fertility, foetolethality, specific developmental abnormalities (craniofacial, musculoskeletal, urogenital), tumours at sites of application recorded.

CARCINOGEN

	US - Rhode Island Hazardous Substance List	IARC	C
MITOMYCIN C	US Environmental Defense Scorecard Recognized Carcinogens	Reference(s)	P65
MITOMYCIN C	US Environmental Defense Scorecard Suspected Carcinogens	Reference(s)	P65
VPVB_(VERY~	US - Maine Chemicals of High Concern List	Carcinogen	CA Prop 65

Section 12 - ECOLOGICAL INFORMATION

This material and its container must be disposed of as hazardous waste.

Ecotoxicity

Ingredient	Persistence: Water/Soil	Persistence: Air	Bioaccumulation	Mobility
mitomycin C	LOW	LOW	LOW	HIGH

Section 13 - DISPOSAL CONSIDERATIONS

US EPA Waste Number & Descriptions

B. Component Waste Numbers

When mitomycin C is present as a solid waste as a discarded commercial chemical product, off-specification species, as a container residue, or a spill residue, use EPA waste number U010 (waste code T).

Disposal Instructions

All waste must be handled in accordance with local, state and federal regulations.

¦ Legislation addressing waste disposal requirements may differ by country, state and/ or territory. Each user must refer to laws operating in their area. In some areas, certain wastes must be tracked.

A Hierarchy of Controls seems to be common - the user should investigate:

· Reduction

· Reuse
· Recycling
· Disposal (if all else fails)
This material may be recycled if unused, or if it has not been contaminated so as to make it unsuitable for its intended use. Shelf life considerations should also be applied in making decisions of this type. Note that properties of a material may change in use, and recycling or reuse may not always be appropriate.
DO NOT allow wash water from cleaning equipment to enter drains. Collect all wash water for treatment before disposal.
· Antineoplastic (cytotoxic) wastes must be packed directly, ready for incineration, into color-coded, secure, labelled, leak-proof containers sufficiently robust to withstand handling without breaking, bursting or leaking.
· Containers of special design are available for particular needs (such as disposal of sharps) and should be used.

Section 14 - TRANSPORTATION INFORMATION

DOT:
Symbols: None Hazard class or Division: 6.1
Identification Numbers: UN3249 PG: II
Label Codes: 6.1 Special provisions: T3, TP33
Packaging: Exceptions: 153 Packaging: Non- bulk: 212
Packaging: Exceptions: 153 Quantity limitations: 5 kg
Passenger aircraft/rail:
Quantity Limitations: Cargo 5 kg Vessel stowage: Location: C
aircraft only:
Vessel stowage: Other: 40
Hazardous materials descriptions and proper shipping names:
Medicine, solid, toxic, n.o.s.

Air Transport IATA:

ICAO/IATA Class: 6.1 ICAO/IATA Subrisk: None
UN/ID Number: 3249 Packing Group: II
Special provisions: A3
Cargo Only
Packing Instructions: 100 kg Maximum Qty/Pack: 25 kg
Passenger and Cargo Passenger and Cargo
Packing Instructions: 676 Maximum Qty/Pack: 669
Passenger and Cargo Limited Quantity Passenger and Cargo Limited Quantity
Packing Instructions: 1 kg Maximum Qty/Pack: Y644
Shipping Name: MEDICINE, SOLID, TOXIC, N.O.S.(CONTAINS
MITOMYCIN C)

Maritime Transport IMDG:

IMDG Class: 6.1 IMDG Subrisk: None
UN Number: 3249 Packing Group: II
EMS Number: F-A , S-A Special provisions: 221
Limited Quantities: 500 g
Shipping Name: MEDICINE, SOLID, TOXIC, N.O.S.
(contains mitomycin C)

Section 15 - REGULATORY INFORMATION

mitomycin C (CAS: 50-07-7) is found on the following regulatory lists;
"Canada - Saskatchewan Environmental Persistent or Chronic Hazardous Substances","Canada Non-Domestic Substances List (NDSL)","International Agency for Research on Cancer (IARC) - Agents Reviewed by the IARC Monographs","US - California Air Toxics ""Hot Spots"" List (Assembly Bill 2588) Substances which need not be reported unless manufactured by the facility","US - California Occupational Safety and Health Regulations (CAL/OSHA) - Hazardous Substances List","US - California Proposition 65 - Carcinogens","US - California Proposition 65 - No Significant Risk Levels (NSRLs) for Carcinogens","US - Maine Chemicals of High Concern List","US - Massachusetts Oil & Hazardous Material List","US - Minnesota Hazardous Substance List","US - New Jersey Right to Know Hazardous Substances","US - Pennsylvania - Hazardous Substance List","US - Rhode Island Hazardous Substance List","US - Vermont Hazardous Constituents","US - Vermont Hazardous wastes which are Discarded Commercial Chemical Products or Off-Specification Batches of Commercial Chemical Products or Spill Residues of Either","US - Washington Dangerous waste constituents list","US - Washington Discarded Chemical Products List - ""U"" Chemical Products","US Department of Transportation (DOT) List of Hazardous Substances and Reportable Quantities - Hazardous Substances Other Than Radionuclides","US DOE Temporary Emergency Exposure Limits (TEELs)","US List of Lists - Consolidated List of Chemicals Subject to EPCRA, CERCLA and Section 112(r) of the Clean Air Act","US RCRA (Resource Conservation & Recovery Act) - Hazardous Constituents - Appendix VIII to 40 CFR 261","US RCRA (Resource Conservation & Recovery Act) - List of Hazardous Wastes","US SARA Section 302 Extremely Hazardous Substances","US Toxic Substances Control Act (TSCA) - Inventory","US TSCA Section 12(b) - List of Chemical Substances Subject to Export Notification Requirements","US TSCA Section 5(a)(2) - Significant New Use Rules (SNURs)"

Section 16 - OTHER INFORMATION

ND
Substance CAS Suggested codes mitomycin C 50- 07- 7 Carc3; R40 Mut3; R68 Rep3; R63 T; R25

Germany Hazard classification and labelling of medicines with antineoplastic effects (ATC Code L01 and L02)

INN CAS Danger CMR effects CMR effects Other
Cat 1&2 Cat 3
Mitomycin C 50- 07- 7 T R 45 R 46 R 25 R
36/37/38

Reasonable care has been taken in the preparation of this information, but the author makes no warranty of merchantability or any other warranty, expressed or implied, with respect to this information. The author makes no representations and assumes no liability for any direct, incidental or consequential damages resulting from its use. For additional technical information please call our toxicology department on +800 CHEMCALL.

■ Classification of the preparation and its individual components has drawn on official and authoritative sources as well as independent review by the Chemwatch Classification committee using available literature references.
A list of reference resources used to assist the committee may be found at:
www.chemwatch.net/references.

■ The (M)SDS is a Hazard Communication tool and should be used to assist in the Risk Assessment. Many factors determine whether the reported Hazards are Risks in the workplace or other settings. Risks may be determined by reference to Exposures Scenarios. Scale of use, frequency of use and current or available engineering controls must be considered.

Issue Date: Jul-28-2008

Print Date:Apr-8-2011

ScienceLab.com
Chemicals & Laboratory Equipment

Health	3
Fire	1
Reactivity	0
Personal Protection	E

Material Safety Data Sheet
Mitomycin c MSDS

Section 1: Chemical Product and Company Identification

Product Name: Mitomycin c

Catalog Codes: SLM1303

CAS#: 50-07-7

RTECS: CN0700000

TSCA: TSCA 8(b) inventory: Mitomycin c

CI#: Not available.

Synonym: 7-Amino-9-alpha-methoxymitosane; Ametycin; Ametycine; Mitocin-C; Mitomycin; Mitomycin-C; Mitomycinum; Mutamycin; Mytomycin

Chemical Name: Azirino(2',3':3,4)pyrrolo(1,2-a)indole-4,7-dione,6-amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methyl-, carbamate (ester)

Chemical Formula: C15H18N4O5

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Mitomycin c	50-07-7	100

Toxicological Data on Ingredients: Mitomycin c: ORAL (LD50): Acute: 30 mg/kg [Rat]. 23 mg/kg [Mouse].

Section 3: Hazards Identification

Potential Acute Health Effects:
Very hazardous in case of ingestion. Hazardous in case of skin contact (irritant), of eye contact (irritant), of inhalation. Severe over-exposure can result in death.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Classified 2B (Possible for human.) by IARC. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to blood, kidneys, lungs, liver, bone marrow. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.

Skin Contact:
In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

Ingestion:
If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: May be combustible at high temperature.

Auto-Ignition Temperature: Not available.

Flash Points: Not available.

Flammable Limits: Not available.

Products of Combustion: These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...).

Fire Hazards in Presence of Various Substances:
Slightly flammable to flammable in presence of heat. Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

Fire Fighting Media and Instructions:
SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special Remarks on Fire Hazards: As with most organic solids, fire is possible at elevated temperatures

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill: Use appropriate tools to put the spilled solid in a convenient waste disposal container.

Large Spill:
Poisonous solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Eliminate all ignition sources. Call for assistance on disposal.

Section 7: Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe dust. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, acids, alkalis.

Storage:
Keep container tightly closed. Keep container in a cool, well-ventilated area. Sensitive to light. Store in light-resistant containers.

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits: Not available.

Section 9: Physical and Chemical Properties

Physical state and appearance: Solid. (Powdered solid. Crystalline powder.)

Odor: Not available.

Taste: Not available.

Molecular Weight: 334.33 g/mole

Color: Bluish-grey Blue-Violet.

pH (1% soln/water): Not available.

Boiling Point: Not available.

Melting Point: >360°C (680°F)

Critical Temperature: Not available.

Specific Gravity: Not available.

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: The product is more soluble in water; log(oil/water) = -0.4

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol, diethyl ether, acetone.

Solubility:

Soluble in cold water, methanol, acetone. Partially soluble in diethyl ether. Soluble in Butyl Acetate, Cyclohexanone. Slightly soluble in Benzene, Carbon Tetrachloride. Practically insoluble in Petroleum Ether.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Excess heat, incompatible materials

Incompatibility with various substances: Reactive with oxidizing agents, acids, alkalis.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity: Incompatible with oxidizing agents, strong bases, strong acids.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Inhalation. Ingestion.

Toxicity to Animals: Acute oral toxicity (LD50): 23 mg/kg [Mouse].

Chronic Effects on Humans:
CARCINOGENIC EFFECTS: Classified 2B (Possible for human.) by IARC. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. May cause damage to the following organs: blood, kidneys, lungs, liver, bone marrow.

Other Toxic Effects on Humans:
Very hazardous in case of ingestion. Hazardous in case of skin contact (irritant), of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May cause adverse reproductive effects and birth defects (teratogenic). Causes sperm abnormalities in animal. May affect genetic material (mutagenic). May cause cancer

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects: Systemic poisoning can occur from inhalation, and oral exposure. However, limited data are available. It is used as a drug to treat cancer, specfically maligant tumors. The clinical (systemic) effects reported are those noted during theraputic (intravenous) use of Mitomycin., including high dose therapy. Skin: Causes skin irritation. Eyes: Causes eye irritation. May cause chemical conjunctivitis. Inhalation: Causes respiratory tract irritation. May cause pulmonary fibrosis and permanent damage. Can produce delayed pulmonary edema. May cause liver damage. Ingestion: May be fatal if swallowed. May cause gastrointestinal tract irritation with stomatitis, nausea, anorexia, vomiting (sometimes bloody), hypermotility, and diarrhea. May cause liver and kidney damage. May affect the blood and cause bone marrow depression. May affect behavior/central nervous system (confusion, dizziness, drowsiness, fatigue, lethargy, weakness). It may affect the heart(congestive heart failure). May cause blurred vision. It also has the potential for pulmonary toxicity and cause pneumonitis, alveolitis and pulmonary edema. Other symptoms may include black, tarry stools, blood in the urine or stools, difficulty urinitating; unusal bleeding or bruising, pinpoint red spots on skin, fever or chills, lower back or side pain; rash, itiching. Chronic Potential Health Effects: Ingestion/Inhalation: Prolonged or repeated ingestion or inhalation may cause liver damage, kidney damage, anorexia, weight loss, vomiting, mild diarrhea, skin rash, alopecia, convulsions, kidney injury, pulmonary toxicity (pneumonitis, alveolitis, pulmonary fibrosis, dyspnea, rales, hemoptysis, cough). May affect the liver, behavior/central nervous system with symtoms similar to acute ingestion. May cause cardiotoxicity. It may also cause damage to the blood/bone marrow (causing a drop in white blood cells, red blood cells, and platelets, anemia).

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are less toxic than the product itself.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 6.1: Poisonous material.

Identification: : Toxic Solid, organic, n.o.s. (Mitomycin) UNNA: 2811 PG: II

Special Provisions for Transport: Not available.

Section 15: Other Regulatory Information

Federal and State Regulations:
California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer, birth defects or other reproductive harm, which would require a warning under the statute: Mitomycin c California prop. 65 (no significant risk level): Mitomycin c: 9e-005 mg/day (value) California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer which would require a warning under the statute: Mitomycin c Connecticut hazardous material survey.: Mitomycin c Illinois toxic substances disclosure to employee act: Mitomycin c Illinois chemical safety act: Mitomycin c New York release reporting list: Mitomycin c Pennsylvania RTK: Mitomycin c Florida: Mitomycin c Minnesota: Mitomycin c Massachusetts RTK: Mitomycin c Massachusetts spill list: Mitomycin c New Jersey: Mitomycin c New Jersey spill list: Mitomycin c Louisiana RTK reporting list: Mitomycin c California Director's List of Hazardous Substances: Mitomycin c TSCA 8(b) inventory: Mitomycin c TSCA 5(a)2 final significant rules: Mitomycin c TSCA 8(a) IUR: Mitomycin c TSCA 12(b) annual export notification: Mitomycin c SARA 302/304/311/312 extremely hazardous substances: Mitomycin c CERCLA: Hazardous substances.: Mitomycin c: 10 lbs. (4.536 kg)

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200). EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC). CLASS D-2A: Material causing other toxic effects (VERY TOXIC).

DSCL (EEC):
R25- Toxic if swallowed. R36/38- Irritating to eyes and skin. R40- Possible risks of irreversible effects. S1/2- Keep locked up and out of the reach of children. S36/37- Wear suitable protective clothing and gloves. S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S46- If swallowed, seek medical advice immediately and show this container or label.

HMIS (U.S.A.):

Health Hazard: 3

Fire Hazard: 1

Reactivity: 0

Personal Protection: E

National Fire Protection Association (U.S.A.):

Health: 3

Flammability: 1

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate. Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

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2 and Bcl-xL reside in the outer mitochondrial wall and inhibit cytochrome c release. The proapoptotic Bcl-2 proteins Bad, Bid, Bax, and Bim may reside in the cytosol but translocate to mitochondria following death signaling, where they promote the release of cytochrome c. Bad translocates to mitochondria and forms a pro-apoptotic complex with Bcl-xL. This translocation is inhibited by survival factors that induce the phosphorylation of Bad, leading to its cytosolic sequestration. Cytosolic Bid is cleaved by caspase-8 following signaling through Fas; its active fragment (tBid) translocates to mitochondria. Bax and Bim translocate to mitochondria in response to death stimuli, including survival factor withdrawal. Activated following DNA damage, p53 induces the transcription of Bax, Noxa, and PUMA. Upon release from mitochondria, cytochrome c binds to Apaf-1 and forms an activation complex with caspase-9. Although the mechanism(s) regulating mitochondrial permeability and the release of cytochrome c during apoptosis are not fully understood, Bcl-xL, Bcl-2, and Bax may influence the voltage-dependent anion channel (VDAC), which may play a role in regulating cytochrome c release. Mule/ARF-BP1 is a DNA damage-activated E3 ubiquitin ligase for p53, and Mcl-1, an anti-apoptotic member of Bcl-2.

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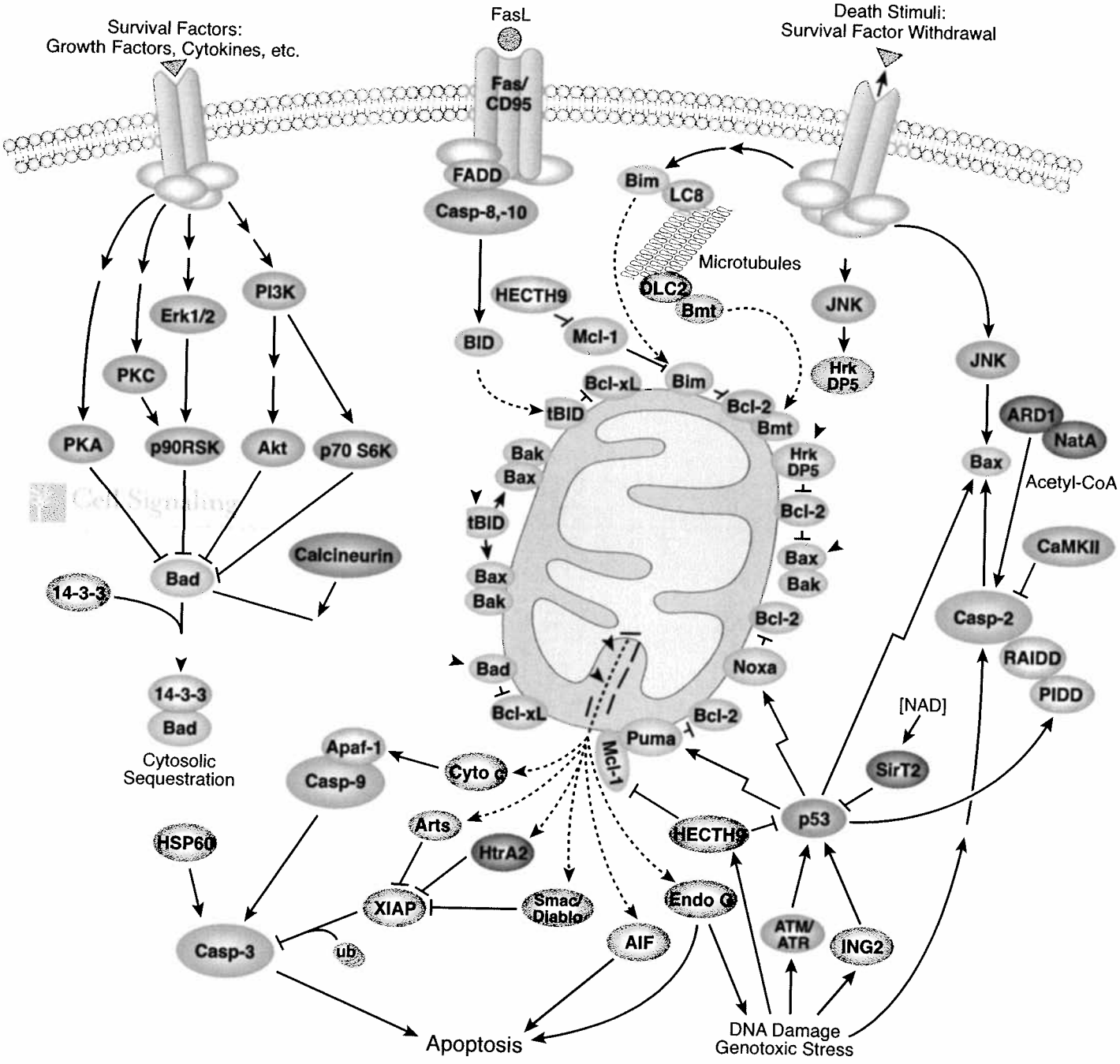
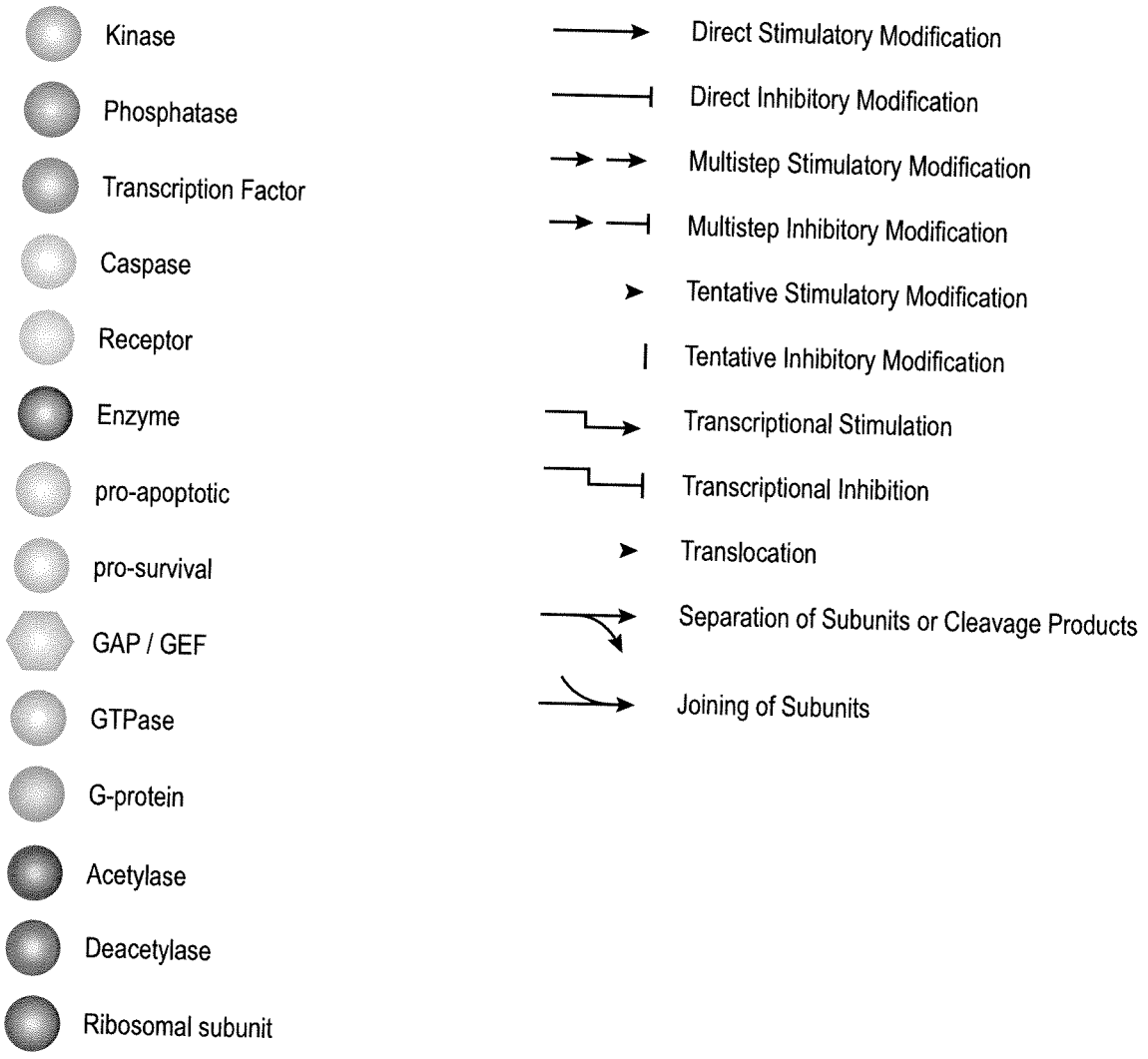
Mitochondrial Control of Apoptosis



Signaling Pathways Key



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Cannabinoids, Endocannabinoids and Cancer

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1. Introduction

1.1 Cannabinoid Function

Endocannabinoids are bioactive lipids that have a range of interesting activities mediated by two G-protein-coupled receptors (CB1 and CB2) and other putative targets [1-3]. The CB1 receptor is present in the central nervous system and mediates the psychotropic effects of exogenous cannabinoids such as Δ^9-tetrahydrocannabinol (THC), the active component of marijuana. In the brain, endocannabinoids and cannabinoids combine with CB1 cannabinoid receptors on axon terminals and regulate ion channel activity and neurotransmitter release [4]. Binding to the CB1 receptor is responsible for the analgesic activity of endocannabinoids as well as many other effects including locomotion and temperature control [5]. The CB2 receptor is present in inflammatory tissues and mediates the anti-inflammatory effects of endocannabinoids and plant-derived cannabinols [6]. Both the CB1 and CB2 receptors couple to Gi and reduce intracellular cAMP levels.

1.2 Biosynthesis and Degradation of Endocannabinoids

Endocannabinoids are synthesized "on demand" by post-synaptic cells and function as retrograde signaling molecules, diffusing back across the synapse to bind with pre-synaptic CB1 receptors, which reduces synaptic transmitter release [7]. The endocannabinoids are primarily produced biosynthetically from phospholipids [8]. The two primary endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The most frequent biosynthetic route for AEA is through the transfer of arachidonic acid (AA) from the *sn*-1 position of phosphatidylcholine (PC) to the nitrogen atom of phosphatidylethanolamine (PE) by *N*-acyl transferase (NAT) to form N-arachidonoyl-phosphatidylethanolamine (NAPE) [8, 9]. NAPE is then converted into AEA in a one-step hydrolysis reaction catalyzed by the NAPE-specific phospholipase D (NAPE-PLD) (Figure 1) [10]. 2-AG is most frequently synthesized through the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) with AA on the *sn*-2 position to diacylglycerol (DAG) by phospholipase C-β (PLC-β). The DAG is then hydrolyzed to 2-AG by diacylglycerol lipase (DAGL) (Figure 2) [7, 11, 12].

The inactivation of endocannabinoids occurs by enzyme-catalyzed hydrolysis to AA; 2-AG is hydrolyzed by monoacylglycerol lipase (MAGL) and AEA is hydrolyzed by fatty acid amide hydrolase (FAAH) (Figure 3) [13, 14]. Endocannabinoids are also substrates for cycloxygenase-2 (COX-2), lipoxygenases (LOXs), and cytochromes P450 (CYP450s) and it is possible that these enzymes also play a role in controlling endocannabinoid levels by oxygenating 2-AG and AEA [15]. Induction of COX-2, LOXs, and CYP450s at sites of

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inflammation or in tumor cells could reduce the levels of naturally occurring anti-inflammatory and anti-proliferative lipid mediators. Furthermore, the products of endocannabinoid metabolism may exert effects that stimulate inflammation or tumor cell development by activating receptors that are distinct from classic receptors or by hydrolysis to products that are known to contribute to both inflammation and tumorigenesis [16]

Although the original focus of endocannabinoid biology was on neurological and psychiatric effects, these molecules are increasingly appreciated for their role in inflammation and cancer. The role of endocannabinoids in cancer has been implied by studies of the effects of exogenous cannabinoids, many derived from the plant *Cannabis sativa*, and synthetic compounds with activity at the CB1 and CB2 receptors (Figure 4). Endocannabinoids inhibit proliferation of cancer cells in culture and *in vivo* [17, 18]. In addition, endocannabinoids inhibit colonic inflammation, and deletion of CB receptors enhances colonic inflammation and cancer [19-21].

2. Cannabinoids and Cancer

2.1 Cannabinoid and Endocannabinoid Mediated Effects

Many laboratories have proposed that cannabinoids and endocannabinoids directly inhibit tumor growth *in vitro* and in animal tumor models through several different pathways. The inhibition of tumor growth and progression of several types of cancers including glioma, glioblastoma, breast cancer, prostate cancer, thyroid cancer, colon carcinoma, leukemia, and lymphoid tumors have been demonstrated by natural and synthetic cannabinoids, endocannabinoids, endocannabinoid analogs, endocannabinoid transport inhibitors, and endocannabinoid degradation inhibitors. Several different mechanisms have been implicated in the anti-tumorigenic actions of endocannabinoids and include cytotoxic or cytostatic effects, apoptosis induction, and anti-metastatic effects such as inhibition of neo-angiogenesis and tumor cell migration [22]. These effects are dependent on CB1, CB2, transient receptor potential vanilloid type 1 (TRPV1), or are receptor-independent based on the cannabinoid or endocannabinoid and the tissue or tumor cell.

Endocannabinoid levels are finely modulated under physiological and pathological conditions. A transient increment appears to be an adaptive reaction to restore homeostasis when this is acutely and pathologically perturbed. However, in some chronic conditions, the alteration of the endocannabinoid system seems to contribute to the progress and symptoms of the disease. In particular, several different types of cancer have abnormally regulated endocannabinoid systems.

2.2 Changes in Endocannabinoid Tone and Signaling in Tumors

Elevated levels of AEA and 2-AG have been reported in several types of tumors when compared with their normal counterparts, specifically in glioblastoma, meningioma, pituitary adenoma, prostate and colon carcinoma, endometrial sarcoma, and in highly invasive human tumor cells [22-27]. The enzymes that synthesize and metabolize the endocannabinoids control their effects by modulating the localized concentrations. A correlation between endocannabinoid metabolizing enzymes, FAAH (for AEA) and MAGL (for 2-AG), and cancer has been investigated in prostate adenocarcinomas. MAGL is elevated in androgen-independent versus androgen-dependent human prostate cancer cell lines, and pharmacological or RNA-interference disruption of MAGL impairs prostate cancer aggressiveness [138]. An increase of FAAH expression in prostate cancer compared to normal prostate tissue samples has been reported [29]. In contrast, in human patients with pancreatic ductal adenocarcinomas a correlation between high FAAH and MAGL levels and survival has been observed [30].

Cannabinoid receptor levels are a major determinant of the effects of endocannabinoids. CB1 receptors show an increase in expression when treated with agonists in several cancer cell lines; however, in normal tissue these agonists decrease CB1 receptor expression [31]. This difference in expression may be a mechanism by which normal cells are protected from the pro-apoptotic and anti-proliferative effects of cannabinoid agonists [22]. It has been shown that THC induces apoptosis in several human cancer cell lines while sparing non-transformed cell lines [32-35].

Cannabinoid receptor expression in tumor cells versus normal cells is an important consideration. Although the mechanisms by which cannabinoid receptor expression is modulated have not been fully investigated, several important studies have revealed critical interactions between cannabinoid receptor expression and cancer. For example, it has been shown that THC induces a CB2 receptor-dependent transcription of the CB1 gene in T cells and T cell lymphoma lines [36]. Up-regulation of the CB1 gene is mediated by IL-4 release and activation of the transcription factor STAT6 [36]. It has been reported that oral administration of specific *Lactobacillus* strains induce CB2 receptor expression in colonic epithelial cells through the NF-κB pathway [37]. In addition, CB1 receptor expression is induced by 17-β-estradiol in human colon cancer cells through an estrogen-receptor dependent mechanism [38]. Chromatin immunoprecipitation studies have demonstrated that the CB1 gene is a transcriptional target of PAX3/FKHR, a chimeric transcription factor found in alveolar rhabdomyosarcoma, where the CB1 receptor is highly overexpressed [39]. Another theory has been presented that alternative spliced isoforms of CB1 (CB1a and CB1b) could reflect differences in its functionality in normal and malignant tissues [40].

The association of CB receptor expression with tumor malignancy and disease outcome in cancer has been studied in several settings. These studies suggest that the role of CB1 and CB2 receptor expression in relation to disease prognosis and outcome is dependent on the specific cancer type. Analyses of astrocytomas demonstrate that 70% of the tumors express CB1 and/or CB2 and the extent of CB2 expression correlates with tumor malignancy [41]. In gliomas, a higher expression of CB2 compared to CB1 has been reported and is related to tumor grade [41]. In addition to tumors, tumor-associated endothelial cells exhibit immunoreactivity for CB receptors similar to that observed in tumor cells [42]. Increased expression of CB1 has been reported in mantle cell lymphoma and of both CB1 and CB2 in non-Hodgkin lymphoma as compared to reactive lymph nodes [43, 44]. In contrast, a greatly reduced expression of CB1, but not CB2, was found in colon carcinoma compared with adjacent normal mucosa [19].

In breast cancer, a correlation between CB2 expression and the histological grade of the tumors as well as other markers, such as estrogen and progesterone receptor levels and the presence of the ERBB2/HER-2 oncogene, has been observed [45]. In prostate cancer, CB1 receptor expression by the human prostate cancer cell lines LNCaP (androgen-sensitive), DU145 and PC3 (androgen-independent) are higher than that seen in normal human prostate epithelial cells [46]. This was confirmed in prostate carcinoma specimens where expression of the CB1 and TRPV1 receptors are up-regulated and correlate with increasing tumor grades [47]. It has also been shown that the level of CB1 in tumor tissue is associated with disease severity at diagnosis and outcome [48]. In pancreatic tumors high CB1 receptor expression is associated with a shorter survival time (median 6 months) than low CB1 expression (median 16 months) in humans [30]. In contrast, in hepatocellular carcinoma, over-expression of CB1 and CB2 receptors are correlated with improved prognosis in humans [49].

2.3 Cannabinoid Receptor-Independent Effects

In addition to signaling through cannabinoid receptors, cannabinoids, in particular anandamide and cannabidiol, have CB receptor-independent effects. AEA and other lipids have been shown to activate TRPV1 [50]. AEA has been shown to induce neuroblastoma, lymphoma, and uterine cervix carcinoma cell death through vanilloid receptors [51, 52]. In addition, inhibition of cancer cell invasion through TIMP1, an inhibitor of metalloproteinases, by methanandamide (AM-356), a hydrolysis resistant AEA analog, is mediated by TRPV1 [45]. It has also been proposed that lipid rafts, membrane domains rich in sphingolipids and cholesterol, mediate AEA effects through CB1 signaling [53, 54]. In cholangiocarcinoma, the anti-proliferative and pro-apoptotic action of AEA is facilitated by lipid raft stabilization, ceramide accumulation, and recruitment of FAS and FAS ligand into lipid rafts [55].

Another cellular protein that may be important in CB receptor-independent cell death induced by endocannabinoids is COX-2. COX-2 metabolizes AA to prostaglandins (PGs) and elevated levels of both COX-2 and PGs have been measured in neoplastic tissues. COX-2 is also capable of metabolizing AEA to prostaglandin ethanolamides (PG-EAs) and 2-AG to glycerol prostaglandins (PG-Gs) [56, 57]. AEA inhibits growth and induces apoptosis in the colon carcinoma cell lines HT29, a moderate COX-2 expressor, and HCA7/C29, a high COX-2 expressor [57]. AEA also inhibits growth and induces apoptosis in COX-2 transfected tumorigenic keratinocytes, but has little effect on the very low COX-2 expressing colon carcinoma cells SW480 and HaCaT keratinocytes [58]. Apoptosis induced by AEA in human neuroglioma cells is COX-2 mediated and not affected by antagonists of the cannabinoid receptors or TRPV1 [59]. In human neuroblastoma and C6 glioma cells AEA induces apoptosis through a vanilloid receptor mediated increase in intracellular calcium concentration, which activates COX-2, releases cytochrome *c* and activates caspase 3 [52].

An important molecule for studying cannabinoid receptor-independent effects is cannabidiol. Cannabidiol is a cannabinoid analog that has no activity at CB1 or CB2 receptors and lacks psychotropic effects. Cannabidiol has been shown to inhibit glioma and breast tumor growth *in vitro* and *in vivo* through induction of apoptosis and inhibition of cell migration and angiogenesis, with these effects being independent of CB and TRPV1 receptor activity [60-62]. Cannabidiol reduces the invasiveness of breast cancer cells by inhibiting Id-1, an inhibitor of basic helix-loop-helix transcription factors involved in tumor progression, at the promoter level [63]. A quinone analog of cannabidiol, HU-331, a highly specific inhibitor of topoisomerase II, has been reported to have high efficacy against human cancer cell lines *in vitro* and against tumor grafts in nude mice [64]. HU-331 also inhibits angiogenesis by directly inducing apoptosis of vascular endothelial cells without changing the expression of pro- and anti-angiogenic cytokines and their receptors [65].

Cannabinoids may also interfere with the ability of lysophosphatidylinositol (LPI) to bind to GPR55. LPI induces cancer cell proliferation through GPR55 activation by triggering the initiation of ERK, AKT, and calcium mobilization cascades [66]. The activation of these cell proliferation cascades by GPR55 has been verified using siRNA to block LPI signaling through GPR55 [66]. In addition, pretreatment of breast and prostate cancer cells with cannabidiol or Rimonabant (SR141716A), a CB1 antagonist that also binds to GPR55, blocks the ability of LPI to induce cell proliferation through GPR55 [66].

3. CB1 and CB2 Mediated Anti-proliferative and Apoptotic Effects of Cannabinoids

3.1 Cannabinoid Modulation of Cell Cycle Regulation

Cannabinoids have been shown to cause cell cycle arrest in various cancer cell lines. AEA arrests the proliferation of MDA-MB-231 human breast cancer cells in the S phase of the cell cycle through a loss in Cdk2 activity, up-regulation of p21waf, and a reduced formation of the active complex cyclin E/Cdk2 [67]. AEA arrests cells in S phase through activation of Chk1 and Cdc25A proteolysis, which prevents activation of Cdk2 through dephosphorylation of Thr14/Tyr15, critical inhibitory residues on Cdk2 [67]. THC inhibits breast cancer cell proliferation by blocking the progression of the cell cycle in the G2/M phase through the down-regulation of Cdc2 in a CB2 receptor-dependent manner [68]. However, CB2-selective antagonists significantly, but not totally, prevent these effects, suggesting a contribution of a CB2 receptor-independent mechanism [68]. The CB1 and CB2 agonist WIN-55,212-2 causes LNCaP human prostate cancer cell arrest in the G0/G1 phase of the cell cycle [69]. Activation of ERK1/2, induction of p27/KIP1, and inhibition of cyclin D sustain the arrest [69].

Importantly, G0/G1 arrest enhances the Bax/Bcl-2 ratio and activates caspases, resulting in an induction of apoptosis. WIN-55,212-2 treatment of LNCaP cells also causes a dose-dependent decrease in the expression of cyclin D1, cyclin D2 and cyclin E, as well as cdk2, cdk4 and cdk6, pRb and its molecular partner, the transcription factor E2F [69]. WIN-55,212-2 causes a dose-dependent decrease in the protein expression of DP-1 and DP-2, which form heterodimeric complexes with E2F essential for activity [69]. THC administration also elicits G0/G1 cell cycle blockade in glioblastoma cells through the suppression of E2F1 and Cyclin A and the up-regulation of the cell cycle inhibitor p16(INK4A) [70].

3.2 Induction of Apoptosis by Cannabinoids

THC has been shown to induce apoptosis via CB1 inhibition of RAS-MAPK and PI3K-AKT survival signaling and induction of BAD-mediated apoptosis in colorectal cancer cells [71]. CB1 also reduces cyclic AMP-dependent protein kinase A signaling leading to down-regulation of the anti-apoptotic factor survivin [45]. Survivin over-expression is associated with poor clinical outcomes and reduced tumor apoptosis in patients with colorectal cancer [73, 74]. Survivin is an attractive target for pharmacological modulation because it is over-expressed in most human tumors but is present in very small amounts in normal adult tissues [74]. A direct link between CB1 activation and decreased survivin expression has been established through treatment of SW-480 cells with AM-356, a CB1 receptor agonist [19].

Activation of CB1 or CB2 receptors has been shown to stimulate *de novo* synthesis of ceramide in human tumors including glioma, leukemia, and pancreatic, and DLD-1 and HT29 colorectal cancer cells [75-77]. Ceramide is a pro-apoptotic lipid that causes up-regulation of the stress protein p8 and several downstream stress-related genes expressed in the endoplasmic reticulum including ATF-4, CHOP, and TRB3 [78]. Ceramide also causes prolonged activation of the Raf1/extracellular signal-regulated kinase cascade, inhibition of Akt, c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase [33,59,79-83]. In DLD-1 and HT29 colorectal cancer cells, CB1 and CB2 receptor activation leads to increased ceramide levels, whereas CB1 and CB2 receptor-induced apoptosis is prevented by the pharmacologic inhibition of *de novo* ceramide synthesis [77]. The synthesis of ceramide appears to be mediated in part by TNFα; knockdown of TNFα abrogates the ceramide increase and prevents the apoptotic effect induced by cannabinoid receptor activation [77].

A role for Bcl-2 family members, such as Bad, has also been hypothesized in cannabinoid-dependent apoptosis [81]. Pro-apoptotic effects may rely also on a CB1 receptor-independent stimulation of sphingomyelin breakdown [84]. In lymphoma and leukemia cell lines, CB agonists such as THC and WIN-55,212-2 induce CB-dependent apoptosis through ceramide accumulation and caspase activation via the p38MAPK signaling pathway, down-regulation of the RAF1/MAPK pathway, and translocation of BAD to mitochondria [85, 86]. A common event in cannabinoid-induced apoptosis is the depolarization of mitochondria via cytochrome c release [85-87]. CB agonists have been reported to be mitochondrial inhibitors, since they decrease oxygen consumption and mitochondrial membrane potential while increasing mitochondrial hydrogen peroxide production, thus inducing apoptosis [88].

3.3 Inhibition of Tumor Proliferation by Cannabinoids

Cannabinoids inhibit the proliferation of various tumor cells through the inhibition of proliferative and oncogenic pathways such as adenylyl cyclase and cAMP/protein kinase A pathway, cell cycle blockade with induction of the cyclin-dependent kinase inhibitor $p27^{kip1}$, decrease in epidermal growth factor receptor (EGF-R) expression and/or attenuation of EGF-R tyrosine kinase activity, decrease in the activity and/or expression of nerve growth factor, prolactin or vascular endothelial growth factor tyrosine kinase receptors [32, 89-93].

AEA inhibits breast cancer cell proliferation through down-regulation of the prolactin receptor, *brca1* gene product, and the high affinity neurotrophins receptor *trk* [89, 93]. The anti-proliferative effect of AEA is proportional to the degree of hormone dependency of the cell lines and the mechanism relies on the inhibition of the cAMP-dependent PKA pathway [93]. Several intraepithelial or invasive prostatic cancers show increased expression of EGF-R, EGF and transforming growth factor α (TGFα) [91]. AEA inhibits the EGF-induced proliferation of DU145 and PC3 prostate cancer cells, as well as of androgen-stimulated LNCaP cells, via G1 arrest and down-regulation of EGF-R [91]. These effects are CB1-mediated [91]. Similar growth arrest and receptor modulation by AEA are observed in prolactin and nerve growth factor-stimulated DU145 cells [92-94]. Treatment of LNCaP cells with WIN-55,212-2 results in decreased proliferation, androgen receptor expression, VEGF protein expression, and secreted levels of PSA, a glycoprotein androgen receptor-regulated protein that is a marker of prostate cancer progression [46]. The antagonistic effect of endocannabinoids on growth factor-induced proliferation has also been reported in glioma [95].

3.4 Inhibition of Tumor Neovascularization by Cannabinoids

Cannabinoids have been shown to inhibit tumor growth by lowering vascular density in tumors. Several cannabinoids that bind to CB1 and/or CB2 receptors, including WIN-55,212-2, HU-210, JWH-133, and THC, have been shown to inhibit vascular endothelial cell survival and migration as part of their antiangiogenic action [96]. Cannabinoids cause a lower distribution of CD31-positive cells, a common angiogenesis marker, in experimental tumor xenografts from glioma, melanoma and nonmelanoma skin cancer, and lung tumor cells [32, 96-98]. Met-fluoro-anandamide (Met-F-AEA), a metabolically stable analog of AEA, has been demonstrated to reduce the sprout number and length of endothelial cell spheroids, inhibit capillary-like tube formation *in vitro*, and suppress angiogenesis in an *in vivo* chick chorioallantoic membrane assay [99]. Furthermore, experimental tumors from animals treated with cannabinoids have been shown to exert a vascular network that is small, undifferentiated, and impermeable giving tumors a paler appearance when compared to controls [90, 96].

In addition to the direct inhibition of vascular endothelial cell migration and survival, cannabinoids decrease the expression of proangiogenic factors in tumors. Several studies

have revealed that cannabinoids have an effect on the expression of VEGF, which is one of the major cancer cell-released chemoattractants in tumor neovascularization [100]. Met-F-AEA has been shown to decrease levels of VEGF and VEGFR-1 in K-ras-transformed thyroid cells and in experimental tumors of xenografted nude mice [90]. In skin carcinoma mouse models, JWH-133 and WIN-55,212-2 inhibit vascular hyperplasia, which is associated with a reduced mRNA expression of VEGF [32]. THC suppresses the release of VEGF in non–small cell lung cancer (NSCLC) cells [98]. JWH-133 mediates decreased expression of proangiogenic factors related to VEGF signaling in mouse gliomas including VEGF-A, VEGF-B, and hypoxia-inducible factor 1α (HIF-1α), which is the main transcription factor responsible for VEGF expression [101]. JWH-133 down-regulates connective tissue growth factor and heme oxygenase-1, genes known to be regulated by VEGF, as well as the VEGF-related factors, inhibitor of differentiation-3 (Id-3), midkine, and the angiopoietin receptor tyrosine kinase with immunoglobulin-like and epidermal growth factor (EGF)-like domains 1 (Tie-1) [101, 102]. In contrast, JWH-133 induces the expression of type I procollagen 1α chain, a matrix metalloproteinase (MMP) substrate related to matrix remodeling during angiogenesis [101, 103].

In vivo experiments have also demonstrated that JWH-133 and WIN-55,212-2 decrease the mRNA levels and autophosphorylation activity of EGFR in skin tumors [32]. Cannabinoids diminish the expression of angiopoietin-2 (Ang-2) and placental growth factor (PlGF) along with the appearance of narrow capillaries and a decrease of blood vessel size [32]. JWH-133 down-regulates Ang-2, which supports the formation of mature blood vessels, in gliomas and astrocytomas [96, 101].

Angiogenesis involves several proteolytic enzymes. THC down-regulates the proangiogenic factor MMP-2 in human tumor samples from recurrent glioblastoma multiforme and in nude mice xenografted with the C6.9 subclone from rat glioma C6 cells [104]. Importantly, THC does not alter the expression of MMP-2 in the C6.4 subclone, a nonresponder, from rat glioma C6 cells [104]. THC and methanandamide decrease MMP-2 expression *in vitro* in cervical cancer cells accompanied by a reduced invasiveness of the cancer cells [45]. JWH-133 also decreases MMP-2 expression *in vivo* in glioma xenografts and impairs tumor vasculature [96]. Met-F-AEA also inhibits MMP-2 activity in endothelial cells [99].

The effects of cannabinoids on several antiangiogenic factors have also been studied. WIN-55,212-2 and JWH-133 do not have an effect on the expression of thrombospondin-1 and -2, multidomain matrix glycoproteins that inhibit neovascularization, in nude mice xenografted with melanoma carcinoma cells [32]. The effects of cannabinoids on the expression of TIMP-1, an inhibitor of angiogenesis, are dependent on the specific cancer cell line used [103]. In human cervical and lung cancer cells, cannabinoids up-regulate TIMP-1 expression and are anti-invasive [45]. In contrast, THC down-regulates TIMP-1 in glioma cell lines and in human tumor samples from recurrent glioblastoma multiforme patients [105]. JWH-133 also down-regulates TIMP-1 in nude mice xenografted with C6.9 glioma cells [105]. The cannabinoid derivative HU-331 is antiangiogenic through a different mechanism. HU-331 inhibits angiogenesis by directly inducing apoptosis of vascular endothelial cells without modulating the expression of pro- and antiangiogenic factors and their receptors [65].

3.5 Effects of Cannabinoids on Tumor Cell Migration

Tumor cell migration is an important step for the spread of cancer [106]. As an initial step, the primary tumor has to enter lymphatic or blood vessels. Migration of cancer cells is initiated by paracrine or endocrine chemoattractants but is also affected by neurotransmitters and other factors.

Among the chemoattractants that trigger migration, cell growth, proliferation, and differentiation, EGF and its receptor, EGFR, play a pivotal role. THC elicits a decrease of EGF-induced migration of NSCLC cells in scratch wound and Transwell migration experiments but has no effect on basal migration [98]. As described earlier, THC action modulates intracellular signaling events downstream of EGFR, such as inhibition of mitogen-activated protein kinases and protein kinase B (Akt) activity [98]. The impact of cannabinoids on EGFR activation appears to be cell type specific. In glioma and lung carcinoma, cannabinoid receptor agonists induce cell proliferation through cannabinoid-induced EGFR signal transactivation [107]. In skin tumors *in vivo*, WIN-55,212-2 and JWH-133 inhibit EGFR activation [32]. AEA inhibits EGFR expression and inhibits EGFR-induced proliferation, through CB1 signaling, in prostate cancer cells [91]. Human astrocytoma cells have no change in EGFR tyrosine phosphorylation when treated with cannabinoids [108].

Neurotransmitters also play a role in regulating cell migration [109]. Cannabinoids have an inhibitory action on norepinephrine-induced cancer cell migration [110]. AEA, HU-210, and the AEA analog docosatetraenylethanolamide (DEA) block the migration of colon carcinoma cells with low CB2 receptor expression while JWH-133 has no effect [110]. AEA and HU-210 activate both cannabinoid receptors, DEA acts as a CB1 receptor agonist, and JWH-133 acts as a CB2 receptor agonist; thus, in colon carcinoma cells, CB1 likely mediates the antimigratory actions of cannabinoids [110].

The pathways involved in CB1-receptor dependent antimigratory effects have been explored in some depth. In breast cancer cells, Met-F-AEA causes a CB1 receptor-dependent antimigratory effect involving the RhoA/Rho-associated coiled coil-containing kinase (RhoA-Rock) system [111, 112]. Met-F-AEA inhibits the activity of the GTPase, RhoA, and causes RhoA to translocate from the cell membrane to the cytosol, which causes alterations in the actin cytoskeleton [112].

Mast cells are a source of chemoattractants and are possible targets of cannabinoids [113]. Cancer cell migration initiated by mast cells is down-regulated by 2-AG and WIN-55,212-2 in the scratch wound healing assay in a CB1-receptor dependent manner [113]. Human glioma cell migration is inhibited by cannabidiol in a receptor-independent manner, as evidenced by the failure of cannabinoid receptor antagonists and pertussis toxin to reverse the antimigratory action of cannabidiol [61]. AM-356 and THC do not affect the basal migration of human cervical and lung cancer cells, implicating a cell type-specific or chemoattractant-dependent regulation of migration by cannabinoids [45]. Thus, cannabinoids are antimigratory in some cancer cell lines but the underlying signaling pathways are not fully elucidated.

3.6 Influence of Cannabinoids on Cancer Cell Adhesion

The adhesive interaction of tumor cells with the surrounding microenvironment is a critical factor in their growth, migration and metastasis. Matrix proteins such as integrins, cadherins, selectins, and cell adhesion molecules of the immunoglobulin superfamily (IgSF CAMs) are integral to the adhesion of tumor cells to the extracellular matrix (ECM). Cannabinoids have been shown to have various effects on the adhesion of tumors cells to the ECM. Met-F-AEA selectively reduces the adhesion of human breast cancer cells to the ECM component collagen type IV in a CB1 receptor-dependent manner *in vitro*, but has no effect on adhesion to fibronectin and laminin [111]. Met-F-AEA does not affect the expression of integrins but it does decrease their affinity for collagen through suppression of phosphorylation of the focal adhesion kinase (FAK) and the pro-oncogenic tyrosine kinase Src [111]. HU-210 does not have a direct effect on FAK phosphorylation in murine neuroblastoma cells [114].

HU-210 instead phosphorylates FAK-related nonkinase (FRNK), which inhibits FAK activity, in a CB1 receptor-dependent manner [114-117].

Cannabinoids influence IgSF CAMs. WIN-55,212-2 blocks the interleukin 1 (IL-1)-induced up-regulation of intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1- two IgSF CAMs- in human glioblastoma and lymphoma cells in a cannabinoid receptor-independent manner [118]. WIN-55,212-2 produces this effect by inhibiting IL-1-induced activation of the transcription factor NFκB, a key regulator in the expression of cell adhesion molecules [118].

3.7 Effects of Cannabinoids on Tumor Cell Invasion

Cancer cell invasion is one of the crucial events in local spreading, growth, and metastasis of tumors. 2-AG inhibits the invasion of androgen-independent prostate cancer cells through CB1 receptor activation [26]. However, the precise mechanism leading to decreased invasiveness by cannabinoids has not been fully elucidated. Several investigations have provided insight into how cannabinoids may achieve their anti-invasive action.

Cannabinoids have been shown to modulate the MMP system, which, in part, leads to their anti-invasive action. MMPs degrade ECM components, an important function in tumor invasion, metastasis, and angiogenesis [119, 120]. MMPs, specifically MMP2- and MMP-9, facilitate tumor invasion by proteolytic degradation of major basement membrane components, such as type IV collagen, laminin, and nidogen [119]. MMP proteolytic activity is inhibited by TIMPs that bind noncovalently in a 1:1 stoichiometric ratio to active MMPs. Higher ECM degradation in invasive and metastatic tumor cells can result from an increased level of MMPs and a decreased level of TIMPs, which causes increased proteolytic activity.

Cannabinoids have been shown to have a direct effect on the MMP system. JWH-133 decreases the expression and activity of MMP-2 in mice xenografted with a rat glioma cell line and human grade IV astrocytoma cells [96]. Met-F-AEA also inhibits MMP-2 activity, leading to an antiangiogenic effect [99]. In addition, THC inhibits MMP-2 expression and cell invasion in glioma cells [104]. Modulation of MMP-2 expression by RNA interference and cDNA overexpression reveals that down-regulation of MMP-2 plays a critical role in THC-mediated inhibition of cell invasion [104]. Cannabinoid-induced inhibition of MMP-2 expression and cell invasion is prevented by blocking ceramide biosynthesis and by knocking down the expression of the stress protein p8 [104]. A concentration-dependent inhibition of MMP-2 by AM-356 and THC in cervical carcinoma cells also occurs, however, it is independent of CB receptor and TRPV1 activation [45].

There is a correlation between high cancer invasiveness and decreased TIMP-1 expression; in addition, the anti-invasive action of several drugs has been associated with elevated TIMP-1 levels [121-127]. In contrast, TIMP-1 up-regulation is associated with poor patient prognosis because TIMP-1 has MMP-independent antiapoptotic properties [128]. The anti-invasive action of AM-356 and THC depends on the induction of TIMP-1 expression in cervical carcinoma and lung cancer cells [45]. The expression of TIMP-1 is stimulated by CB1 and CB2 receptor activation and, in the case of AM-356, by activation of TRPV1 [45]. TIMP-1 expression has also been shown to be modulated by the p38 mitogen-activated protein kinase and the extracellular regulating kinases 1 and 2 (ERK1/2) [45]. In glioma cell lines and primary tumor cells from glioblastoma multiforme tissues, TIMP-1 expression is inhibited by cannabinoids [105]. Instead, the cannabinoid-induced apoptosis is dependent on *de novo* synthesis of ceramide [105]. Thus, cannabinoid action on TIMP-1 expression and the subsequent impact on tumorigenesis depends on tumor type.

3.8 *In Vivo* Effects of Cannabinoids

In vivo studies demonstrate that cannabinoids reduce tumor growth and metastasis as well as cell proliferation and angiogenesis in mice. THC decreases tumor size, number of tumor and lung metastases, and inhibits both cell proliferation and angiogenesis in an animal model of metastatic breast cancer [129]. This inhibition of cell proliferation involves CB2 but not CB1 receptors [129]. The CB2 agonist JWH-133 reduces the size and number of tumors, number and size of lung metastases, inhibits cell proliferation, and decrease angiogenesis in mice injected with different breast cancer cell lines [129, 130]. In CB-17 immunodeficient mice injected with MDA-MB-231 cells, the mixed CB1/CB2 agonist WIN55,212-2 reduces tumor size, decreases the number and size of lung metastases, inhibits proliferation and reduces angiogenesis through activity at both the CB1 and CB2 receptors [130]. Cannabidiol reduces tumor growth and size and decreases the number of lung metastases in mice injected with MDA-MB-231 or 4T1 breast cancer cell lines [62, 131]. AM-356 reduces the number and size of lung tumor nodules in mice injected with TSA-1 mammary carcinoma cell line through CB1 activity [111]. In contrast, the CB1 antagonist Rimonabant decreases tumor size in mice injected with MDA-MB-231 cancer cells [132]. Direct injection of the preferential CB2 agonist JWH-015 reduces tumor growth in athymic nude male mice injected with PC-3 prostate carcinoma cells and this reduction of growth is inhibited by the CB2 receptor antagonist SR144528 [133].

Although many studies have found beneficial effects of cannabinoids in the treatment of cancer, there are several conflicting reports. Systemic administration of THC increases the local tumor size and the number and size of metastasis in mice injected with 4T1 tumor cells into the rear footpads [134]. This effect may be due to the fact that THC suppresses the anti-tumor immune response, which is mediated by CB2 [134]. SCID-NOD mice, which are devoid of anti-tumor immune responses, do not exhibit increases in tumor size or metastasis following THC administration [134].

4. Conclusions

Cannabinoids exert a number of interesting effects that are dependent on the cell line or tumor type. Synthetic cannabinoids and the endocannabinoid system are implicated in inhibiting cancer cell proliferation and angiogenesis, reducing tumor growth and metastases, and inducing apoptosis. Some studies suggest that abnormal regulation of the endocannabinoid system may promote cancer by fostering physiological conditions that allow cancer cells to proliferate and migrate. For this reason, the endocannabinoid is an attractive target for pharmacological intervention in the treatment of cancer. Modulation of the endocannabinoid system to treat cancer may provide a targeted treatment of cancer, which has been shown in several studies that demonstrated selective action of cannabinoids on tumor cells while not having effects on normal cells.

The endocannabinoid system is involved in a complex set of signaling pathways including activity at the CB1, CB2, TRPV1, and GPR55 receptors, and through receptor-independent actions. The complexity of the signaling pathways involved in endocannabinoid action both in normal and malignant tissues offer a significant research obstacle, however, several important pathways have been elucidated. These include modulation of pathways critical to cell proliferation, cell cycle, and apoptosis. The diversity of receptors and signaling pathways that the endocannabinoid system modulates offers an interesting opportunity for the development of specific molecules to perturb the system selectively, as has already been achieved in the development of agonist and antagonists of the CB1, CB2, TRPV1, and GPR55 receptors. In addition, recent work has revealed that COX-2, which is involved in the progression of several types of cancer, modulates endocannabinoid tone at sites of

inflammation [135]. The oxygenation of endocannabinoids by COX-2 or other enzymes may also play a critical role in the influence of endocannabinoids on cancer.

Although there is a strong set of data *in vitro*, in cellular model systems, and in mouse model systems, there is a dearth of clinical data on the effects of cannabinoids in the treatment of cancer in humans. This fact is quite surprising considering the large library of compounds that have been developed and used to study the effects of cannabinoids on cancer in model systems. Despite the lack of preclinical and clinical data, there is a strong agreement that pharmacological targeting of the endocannabinoid system is emerging as one of the most promising new methods for reducing the progression of cancer. In particular, combination therapy utilizing both traditional chemotherapeutics and molecules targeting the endocannabinoid system may be an excellent next generation treatment for cancer.

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Abbreviations

THC	Δ^9-tetrahydrocannabinol
AEA	anandamide
2-AG	2-arachidonoylglycerol
AA	arachidonic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
NAT	by *N*-acyl transferase
NAPE	N-arachidonoyl-phosphatidylethanolamine
NAPE-PLD	NAPE-specific phospholipase D
PIP_2	phosphatidylinositol-4,5-bisphosphate
DAG	diacylglycerol
PLC-β	phospholipase C-β
DAGL	diacylglycerol lipase
MAGL	monoacylglycerol lipase
FAAH	fatty acid amide hydrolase
COX-2	cycloxygenase-2
LOXs	lipoxygenases
CYP450s	cytochromes P450
TRPV1	transient receptor potential vanilloid type 1
AM-356	methanandamide
PGs	prostaglandins
PG-EAs	prostaglandin ethanolamides
PG-Gs	glycerol prostaglandins

LPI	lysophosphatidylinositol
EGF-R	epidermal growth factor receptor
TGFα	transforming growth factor α
Met-F-AEA	Met-fluoro-anandamide
NSCLC	non–small cell lung cancer
HIF-1α	hypoxia-inducible factor 1α
Id-3	inhibitor of differentiation-3
Tie-1	angiopoietin receptor tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 1
MMP	matrix metalloproteinase
Ang-2	angiopoietin-2
PlGF	placental growth factor
Akt	protein kinase B
DEA	docosatetraenylethanolamide
RhoA-Rock	RhoA/Rho-associated coiled coil-containing kinase
IgSF CAMs	cell adhesion molecules of the immunoglobulin superfamily
ECM	extracellular matrix
FAK	focal adhesion kinase
FRNK	FAK-related nonkinase
IL-1	interleukin 1
ERK1/2	extracellular regulating kinases 1 and 2

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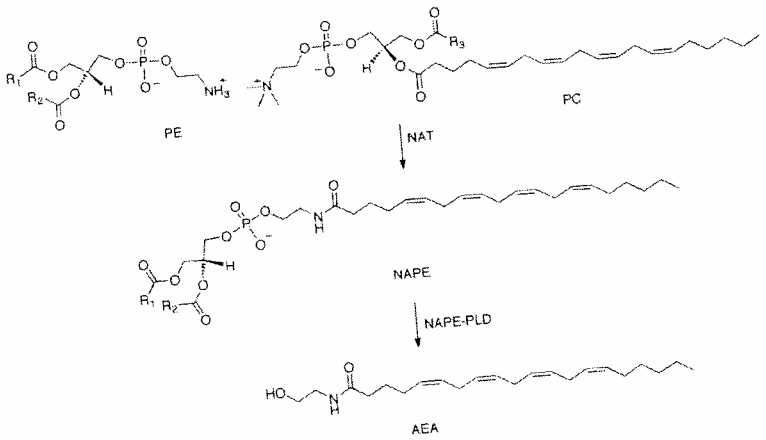

Figure 1. Biosynthesis of AEA

NAT catalyzes the transfer of AA from PC to the ethanolamine of PE to from NAPE. NAPE is then hydrolyzed by NAPE-PLD to form AEA.

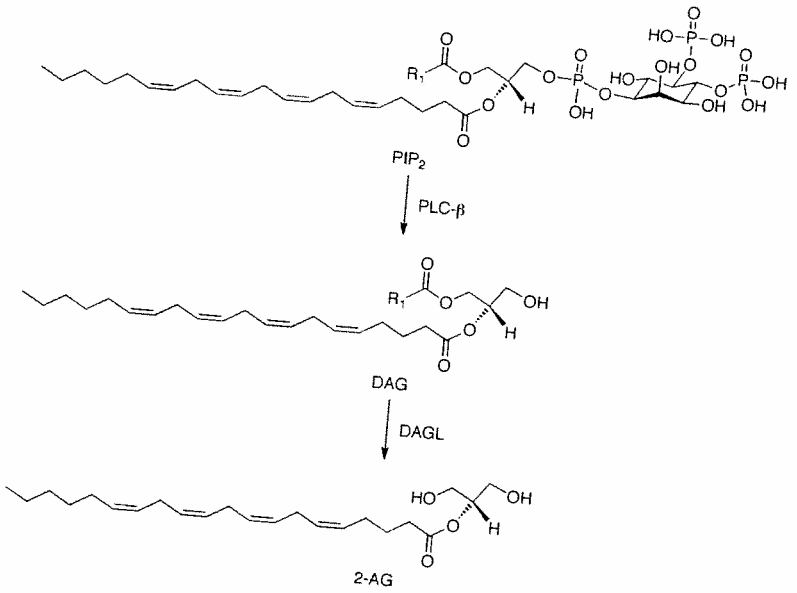

Figure 2. Biosynthesis of 2-AG

PIP_2 is hydrolyzed by PLC-β to form DAG. DAGL then hydrolyzes DAG to form 2-AG.

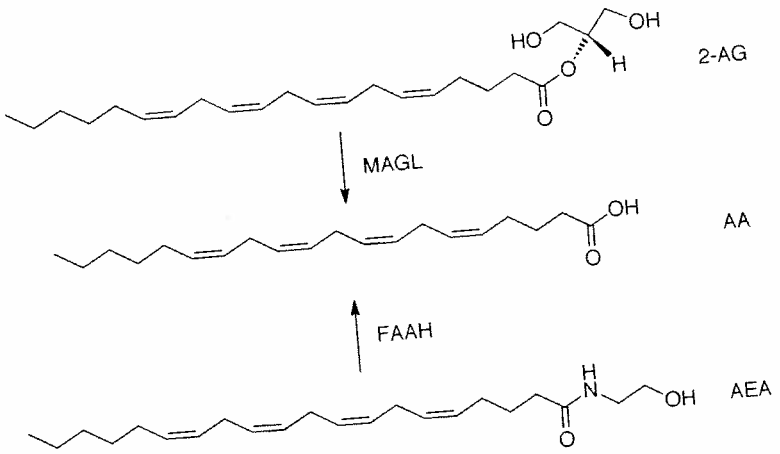

Figure 3. Metabolism of 2-AG and AEA

The endocannabinoids are primarily metabolized by hydrolysis to AA. 2-AG is hydrolyzed by MAGL and AEA is hydrolyzed by FAAH.

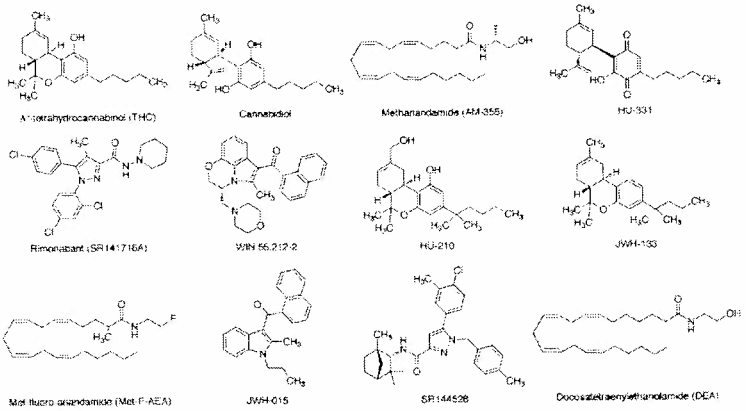

Figure 4.
Structures of compounds used to study the endocannabinoid system.